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RESEARCH ARTICLE

Bat assemblage at a high diversity locality in the Atlantic Forest

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Abstract

The Estação Biológica Santa Lúcia (EBSL) is one of the oldest reserves in Brazil, used as a research site since 1939 by the naturalist Augusto Ruschi. It is an Atlantic Forest fragment, and its fauna and flora have been studied throughout the years. However, its chiropteran fauna remains virtually unknown. Here, we aim to provide a bat species list for EBSL. We installed mist-nets over 19 nights from mid-2009 to mid-2010, with a sampling effort of 20 875.5 m^2.h. We captured 204 specimens, from two families and 22 species. Ecological analysis reveals a medium to high diversity, heavily dominated by frugivorous species. The collector's curve and the estimative of species richness suggests that the chiropteran fauna at EBSL remains partially unknown, and we encourage further inventories.

Keywords

Augusto Ruschi, Chiroptera, fragment, southeastern Brazil, species richness

Introduction

Bats play an important ecological role in their ecosystems, especially in pollination and seed dispersal, feeding on fruits, insects, nectar, and other vertebrates as well as blood (Fenton et al. 1992). Of the 181 species recorded in Brazil (Delgado-Jaramillo

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et al. 2020; Garbino et al. 2020), 83 are known to occur in the state of Espírito Santo (Vela-Ulian et al. in press).

This state is entirely covered by the Atlantic Forest, one of the most diverse and fragmented Brazilian domains, reduced to 11.4% to 16% of its original extension (Ribeiro et al. 2009). Knowledge about bats at the Espírito Santo state began with Maximilian Alexander Philip, the Prince zu Wied-Neuwied. He was a German naturalist explorer, one of the many travelling naturalists who came to the Neotropical Region to catalog the biodiversity (Rocha 1971; Kury 2001). Through his efforts, four bats species were known to occur at Espírito Santo (Schinz 1821; Wied-Neuwied 1826). The nineteenth century had other collections by naturalists, such as Saint-Hilaire and Castelnaeu, until the 1940s, with 11 bat species for the state: *Anoura caudifer*, *Carollia brevicauda*, *Carollia perspicillata*, *Centronycteris maximiliani*, *Desmodus rotundus*, *Glossophaga soricina*, *Myotis nigricans*, *Phyllostomus hastatus*, *Platyrrhinus lineatus*, *Rhynchonycteris naso*, and *Saccopteryx leptura* (Lima 1926; Vieira 1942; Ruschi 1951; Mendes et al. 2010).

The Estação Biológica Santa Lúcia (EBSL) reserve is one of the oldest forest reserves in Brazil. The naturalist Augusto Ruschi conducted researches in this area since 1939, mainly on orchids, hummingbirds and bats (Ruschi-Filho 1972). Ruschi conducted his studies on the chiropterofauna of the state of Espírito Santo between 1950 and 1978, publishing a series of papers documenting each species, accompanied by morphological and behavioral data. He also studied the occurrence of rabies on bats, finding positive cases in 19 species (see Ruschi 1978). Following his efforts, Espírito Santo was found to have 37 species of bats. Afterwards, Adriano Peracchi, the first Brazilian specialist in bats, initiated his collections in 1970 in what today is the Floresta Nacional de Goytacazes, reaching a richness of 55 bat species (Peracchi and Albuquerque 1993). Finally, systematic efforts (e.g., Oprea et al. 2007; Vieira et al. 2008; Peracchi et al. 2011) raised to 83 the number of bat species at Espírito Santo state (Vela-Ulian et al. in press).

The EBSL has a convoluted and, even to this day, partially unsolved history. It dates back to, at least, 1939, when an area next to the Timbuí River margins was acquired by the Museu Nacional and the Sociedade de Amigos do Museu Nacional. Ten years later, Augusto Ruschi created the Museu de Biologia Prof. Mello Leitão (MBML), with an acquisition of a second area, bordering the first. These areas were later contested by the Espírito Santo government, due to some irregularities with the paperwork. It endured until 1980, when ownership of the land by the Universidade Federal do Rio de Janeiro (UFRJ) was recognized, with an area of 156 ha. However, a certain imprecision concerning the name, area and perimeter remained. Therefore, MBML decide to name the union of four contiguous areas as "Estação Biológica Santa Lúcia". The first is the 156 ha aforementioned area, belonging to UFRJ. The second is a 53 ha area belonging to IPHAN (Instituto do Patrimônio Histórico e Artístico Nacional), acquired by Ruschi through the Brazilian State in 1983. A third area, with 129 ha, belongs to the Sociedade de Amigos do Museu Nacional, and was donated

to Ruschi at 1972. Finally, there are 103 ha of preserved vacant land, summing up to 440 ha. However, the perimeter is still undefined (Mendes and Padovan 2000).

A number of studies have been conducted at EBSL, including entomological (Azevedo et al. 2003; Barbosa et al. 2014), botanical (Goldenberg and Reginato 2006; Kollmann 2006; Saiter and Thomaz 2014), ornithological (Ruschi 1977; Simon 2000), and mammalian (Passamani 2000; Passamani et al. 2000; Paresque et al. 2004). However, apart from a few isolated records (e.g., Zortéa and Brito 2000; Redondo et al. 2008; Velazco and Patterson 2008), there has been no systematic effort to collect bats at EBSL. Herein, we provide an updated list of bat species present at EBSL.

Materials and methods

Study site

The Estação Biológica Santa Lúcia (EBSL) reserve is a forest fragment located 7 km southeast from the center of the Santa Teresa city, Espírito Santo state, southeastern Brazil (19°57'55"S, 040°32'23"W) (Fig. 1). The EBSL encompasses about 440 hectares of Atlantic Forest, with many trails available. The vegetation is Montane Ombrophilous Dense Forest (Veloso and Goés-Filho 1982), with signs of anthropic action (Mendes and Padovan 2000). There are several disturbances at EBSL, especially agricultural activity. There is significant garbage disposal in the Timbuí River, the main water course crossing EBSL (Mendes and Padovan 2000), and there is an abundant and constant presence of domestic dogs (Srbek-Araujo and Chiarello 2008).

There are many forest fragments within the Santa Teresa city perimeter, most of them of small size. The EBSL is contained in a fragment located in the southeastern region, almost at the boundary with Santa Leopoldina city. There are two conservation units close to EBSL: the Reserva Biológica Augusto Ruschi (3 598 ha), about 5 km north, and Área de Proteção Ambiental do Pico de Goiapaba-Açú (3 740 ha), approximately 5 km northeast. The majority of the forest fragments are contained within the Reserva da Biosfera da Mata Atlântica (Fig 1). Climate is subtropical humid (Cfa of Köppen), with mean annual temperature of 19.9 °C, ranging from 14.3 to 26.2 °C (Thomaz and Monteiro 1997), and mean annual precipitation of 1 824 mm (SNIRH 2018). The area has a cold and dry season between April and September, and a hot and wet season between October and March, with mean precipitation of 587 mm and 1 237 mm, respectively (SNIRH 2018).

Sampling and analysis

We captured bats with mist nets during 19 nights between May to October 2009, and February to June 2010, which were opened from dusk to dawn (18:00 to 6:00 h) in five different trails (Table 1), during different moon phases and both rainy and dry seasons. We sampled over the course of one to four nights each month, always

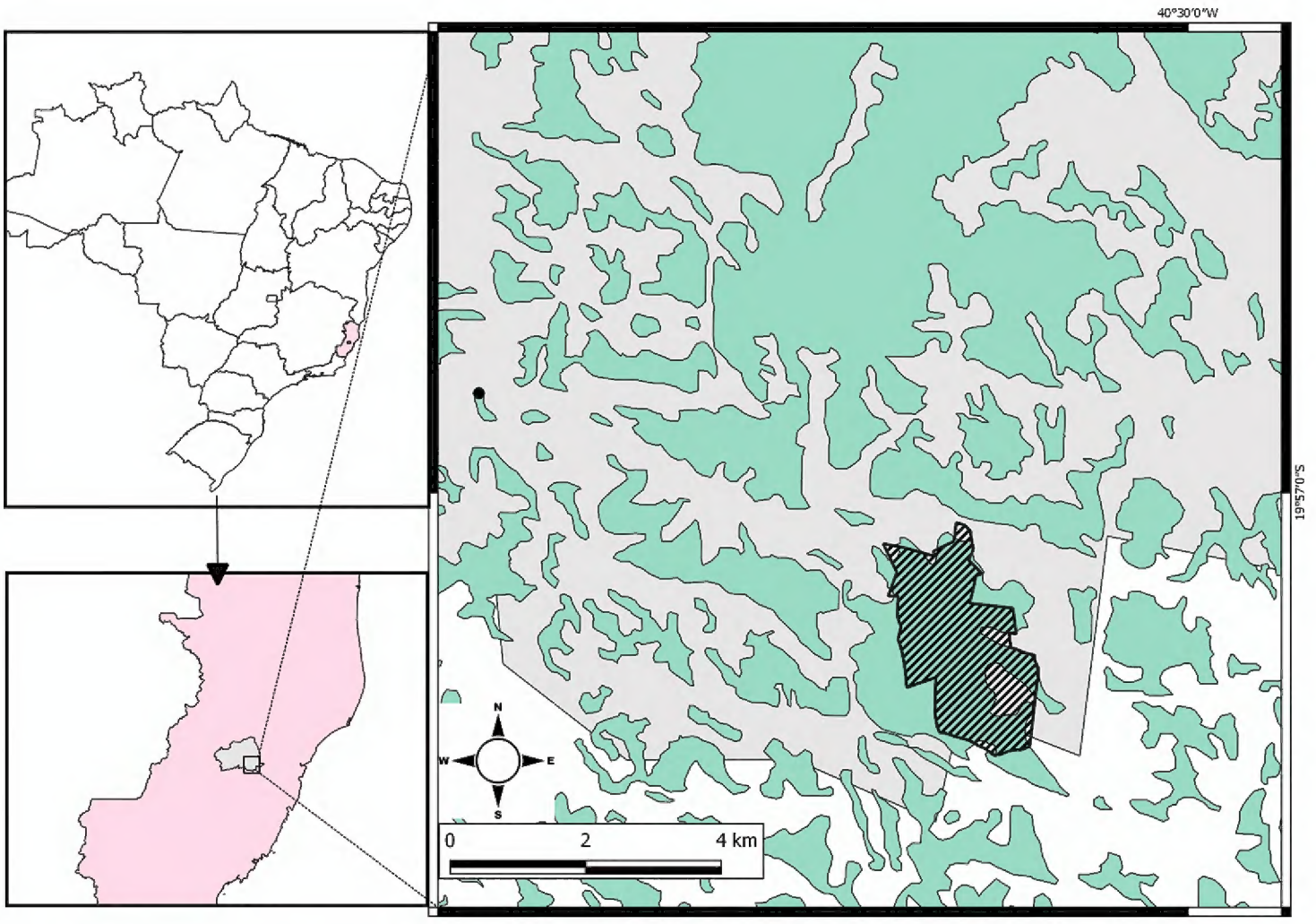

Figure 1. Estação Biológica Santa Lúcia (EBSL) location in southeastern Brazil. The state of Espírito Santo is denoted in pink, and Santa Teresa city in gray. EBSL is represented by the hatched area, with the center of Santa Teresa depicted by the black point. Several forest fragments, depicted in green, surround EBSL.

Table 1. Sampled trails at Estação Biológica Santa Lúcia (EBSL), southeastern Brazil.

Trail	Coordinates	Altitude (m)	Nights
Indaiá-Açú	19°58'00"S, 040°32'17"W	703	4
Rio (near Augusto Ruschi grave)	19°58'23"S, 040°31'44"W	657	7
Rio (near lodgings)	19°57'55"S, 040°31'38"W	648	4
Sagui	19°58'24"S, 040°32'05"W	813	1
Tapinoã	19°58'08"S, 040°31'43"W	708	3

choosing a different trail each night. All mist nets had a height of 3 m, while the length varied from 6 to 15 m (Table 2). Netting effort was 18 466.5 m^2.h, calculated following Straube and Bianconi (2002). Capture efficiency was calculated as the ratio of number of captures by the sampling effort. Identifications followed Gardner (2008) and Díaz et al. (2016).

After capture, we assessed individual weight, sex, reproductive condition, forearm length and feeding guild based on the categories proposed by Schnitzler and Kalko (2001). The first of each species, and those with uncertain identification on the field were collected as vouchers, under license 14592-1937 (IBAMA). The vouchers were first fixed in formaldehyde, then fluid-preserved in 70% alcohol, with the skull removed and cleaned and liver tissue extracted. Vouchers

Table 2. Number of mist nets used each night, by length, with start and finishing hours, and the resulting sampling effort for bat capturing in an area of Atlantic Forest, southeastern Brazil. All mist nets had a 3 m height.

Nights	Trail	Length (m)					Start	Finish	Effort (m^2.h)
		6	7	9	12	15			
09/05/2009	Rio, grave	2	0	2	0	0	17:20	23:45	500.50
10/05/2009	Rio, grave	0	0	4	2	1	17:30	0:10	1300.00
11/07/2009	Rio, lodging	0	0	4	1	1	17:00	0:00	1146.60
14/07/2009	Tapinoã	0	0	3	0	1	17:30	0:30	764.40
15/07/2009	Rio, grave	0	0	4	1	0	17:30	21:30	499.20
16/07/2009	Indaiá-Açú	0	0	5	2	1	17:00	0:00	1528.80
11/08/2009	Rio, lodging	0	0	4	0	0	18:30	0:30	561.60
14/08/2009	Indaiá-Açú	0	0	2	1	1	17:30	0:40	838.50
15/08/2009	Tapinoã	0	1	2	1	1	17:30	0:30	946.40
16/08/2009	Rio, lodging	0	0	3	1	0	17:30	0:30	709.80
27/09/2009	Rio, grave	4	1	2	0	0	18:00	6:00	1528.80
28/09/2009	Indaiá-Açú	4	0	2	0	0	18:00	6:00	1310.40
24/10/2009	Tapinoã	2	2	3	1	0	18:00	5:00	1859.00
27/02/2010	Rio, grave	3	0	0	0	0	18:00	6:00	561.60
28/02/2010	Rio, lodging	4	0	0	0	0	18:00	0:45	421.20
19/03/2010	Sagui	7	0	1	0	0	18:00	3:30	1259.70
03/06/2010	Rio, grave	3	0	0	0	0	17:05	0:15	335.40
04/06/2010	Indaiá-Açú	6	0	0	0	0	17:05	5:40	1177.80
05/06/2010	Rio, grave	6	0	0	0	0	17:25	6:25	1216.80

were deposited in the Mammalian Collection at Universidade Federal do Espírito Santo (UFES-MAM) (see Appendix 1). We built a species accumulation curve, estimated the species richness using the first-order Jackknife estimator, and calculated the species diversity index using the probability of interspecific encounter (PIE) (Hurlbert 1971). PIE is metric that can be interpreted as "what is the probability that two species of a community, taken at random, belong to two different species?" It is the complement of the Simpson's diversity index (D), a metric that asks the chance of, in the aforementioned scenario, being of the same species (PIE = 1 – D), and sometimes is called the Gini-Simpson index (Jost 2007; Chase and Knight 2013). We chose PIE because it is more sensitive to species evenness, and is rather insensitive to species richness, after a certain threshold has been surpassed, around 10 species (Hurlbert 1971). The index ranges from 0 to 1, with 0 a completely uneven community (e.g., a monoculture), and 1 a perfectly even community. A moderately uneven community has values around .75, and a highly uneven has .5 or lower. In order to provide a species diversity comparison, we compiled inventories of bat species conducted at the Atlantic Forest, tracking the study area size, species richness, and calculating PIE through available abundance data. All analysis was conducted using the software R 3.5.3 (R Core Team 2019), with the *vegan* package (Oksanen et al. 2019).

A few sporadic captures were made at EBSL before this study, with vouchers deposited at two collections: the Instituto Nacional da Mata Atlântica (INMA), and the aforementioned UFES-MAM. We checked records of bats in these collections through the speciesLink platform (speciesLink 2020).

Results

We captured a total of 204 individuals, from 22 species of two families, Phyllostomidae and Vespertilionidae (Table 3). Capture efficiency was 0.011 bats/m^2.h. Phyllostomidae accounted for the largest number of species (18 spp.) and individuals, representing over 95% of captured specimens, with Vespertilionidae representing four species and 5% of all individuals captured. Almost 75% of captures were represented by three species, *Carollia perspicillata*, *Artibeus lituratus* and *Artibeus fimbriatus*.

We found slightly more females than males in our study, with 111 females and 85 males (8 specimens lack this data). Regarding the reproductive state, we found seven lactating and one post-lactating female, from the species *Carollia perspicillata*, *Artibeus lituratus*, *Artibeus fimbriatus* and *Sturnira tildae*. We also found 19 pregnant females, from *Artibeus fimbriatus* (N = 5), *Artibeus lituratus* (N = 4), *Artibeus obscurus* (N = 1), *Carollia perspicillata* (N = 8), and *Myotis ruber* (N = 1). We found 13 males with descended testes: *Anoura geoffroyi* (N = 1), *Artibeus lituratus* (N = 5), *Carollia perspicillata* (N = 6), and *Myotis riparius* (N = 1).

Table 3. List of bat species captured at Estação Biológica Santa Lúcia (EBSL), southeastern Brazil, with abundance (by trail and total), and the feeding guild. Museum indicates if the record is based on a voucher deposited in an institution. ANI: Gleaning animalivore, BAI: aerial insectivore, FRU: frugivore, NEC: nectarivore, SAN: sanguivore, I: Indaiá-Açú, II: Rio (near Augusto Ruschi grave), III: Rio (near lodgings), IV: Sagui, V: Tapinoã.

Family	Species	Guild	Museum	I	II	III	IV	V	Total
Phyllostomidae	*Micronycteris microtis*	ANI	–	0	0	0	1	0	1
	Micronycteris minuta	ANI	X	–	–	–	–	–	–
	Desmodus rotundus	SAN	X	0	0	2	0	0	2
	Mimon bennettii	ANI	–	0	1	0	0	0	1
	Phyllostomus hastatus	ANI	–	0	0	0	1	0	1
	Trachops cirrhosus	ANI	X	1	0	0	0	0	1
	Anoura caudifer	NEC	X	0	2	0	0	1	3
	Anoura geoffroyi	NEC	X	2	1	1	0	1	5
	Lonchophylla mordax	NEC	X	–	–	–	–	–	–
	Lonchophylla peracchii	NEC	X	0	1	0	0	0	1
	Carollia brevicauda	FRU	X	–	–	–	–	–	–
	Carollia perspicillata	FRU	X	22	25	14	8	26	95
	Rhinophylla pumilio	FRU	X	0	1	2	5	1	9
	Artibeus fimbriatus	FRU	X	3	3	2	3	5	16
	Artibeus lituratus	FRU	X	6	10	10	1	14	41
	Artibeus obscurus	FRU	X	1	4	2	1	0	8
	Dermanura cinerea	FRU	–	0	1	1	0	0	2
	Platyrrhinus lineatus	FRU	X	–	–	–	–	–	–
	Platyrrhinus recifinus	FRU	X	0	0	0	0	1	1
	Sturnira lilium	FRU	X	1	0	2	0	0	3
	Sturnira tildae	FRU	–	0	1	0	0	1	2
	Vampyressa pusilla	FRU	–	1	0	0	1	0	2
Vespertilionidae	*Eptesicus brasiliensis*	BAI	–	0	0	1	0	0	1
	Eptesicus diminutus	BAI	–	–	–	–	–	–	–
	Lasiurus blossevillii	BAI	X	–	–	–	–	–	–
	Myotis nigricans	BAI	X	0	0	1	0	0	1
	Myotis riparius	BAI	–	0	1	2	2	0	5
	Myotis ruber	BAI	–	0	1	0	1	1	3

The species accumulation curve did not reach an asymptote (Fig. 2B). The first-order Jackknife estimated 29.6 species for EBSL, and the species diversity was PIE = .731. We registered five of the ten trophic guilds. Frugivores represented approximately 88% of the captures. The sanguivores represented less than 1% of the captures. We found 18 species based on museum vouchers, with six of them not found in our sampling: *Micronycteris minuta*, *Lonchophylla mordax*, *Carollia brevicauda*, *Platyrrhinus lineatus*, *Eptesicus diminutus*, and *Lasiurus blossevillii* (Table 3).

We found 119 bat species inventories conducted at the Atlantic Forest containing sufficient data to calculate the area PIE index. We excluded 28 of those inventories before analysis, as they lacked the study area size, or were areas sampled more than once, where we considered the higher species richness (see Suppl. material 1: Table S1).

Discussion

The family Phyllostomidae was the best represented in this Atlantic Forest locality, following a trend depicted by many other studies conducted in the Neotropical Region (e.g., Portfors et al. 2000; Esbérard 2003; Sampaio et al. 2003; Laurindo et al. 2016; Novaes et al. 2017). This pattern is explained by two factors: the wide distribution and high number of species, and especially by sampling bias due to using mist nets at ground level (Fleming 1986; Fenton et al. 1992). Employment of mist nets at canopy level and bioacoustics survey are recommended for a more effective sampling of aerial insectivore bats, such as the Emballonuridae, Molossidae and Vespertilionidae (Carvalho et al. 2013; Marques et al. 2016; Gregorin et al. 2017; Hintze et al. 2020).

Frugivores were the most abundant trophic guild in our sample (Table 3). Three species – *Carollia perspicillata*, *Artibeus lituratus* and *Artibeus fimbriatus* – represented almost 75% of all captured individuals, a pattern observed at the Atlantic Forest (e.g., Esbérard 2003; Lima et al. 2016; Novaes et al. 2017). The observed species diversity index (PIE) indicates a moderately uneven community, close to what is expected for an Atlantic Forest locality of similar observed species richness (Fig. 2A). This can be seen in the composition of the community: almost 75% of occurrences are of three species, while the remaining display a similar number of captured individuals (Table 3). Members of the genus *Artibeus* show a preference for *Ficus*, while *Carollia* consume mostly *Piper* fruits. These botanical genus are abundant throughout the Neotropical Region, providing a homogeneous and available food source (Parolin et al. 2016). In addition, the environmental disturbance at EBSL (Mendes and Padovan 2000) could have caused a reduction of ecological niches, especially most specialized ones, such as gleaning animalivores (e.g., *Lophostoma*, *Lonchorhina*, *Chrotopterus*), frugivorous (*Chiroderma*) and specialized nectarivorous (*Lionycteris*), that are known to occur in this region (Woodman and Timm 2006; Scabello et al. 2009; Mendes et al. 2014).

The species accumulation curve did not reach an asymptote (Fig. 2B). Accordingly, the first-order Jackknife estimated a species richness of 30 species, suggesting that our sampling recorded about 73% of the local richness, and the chiropteran

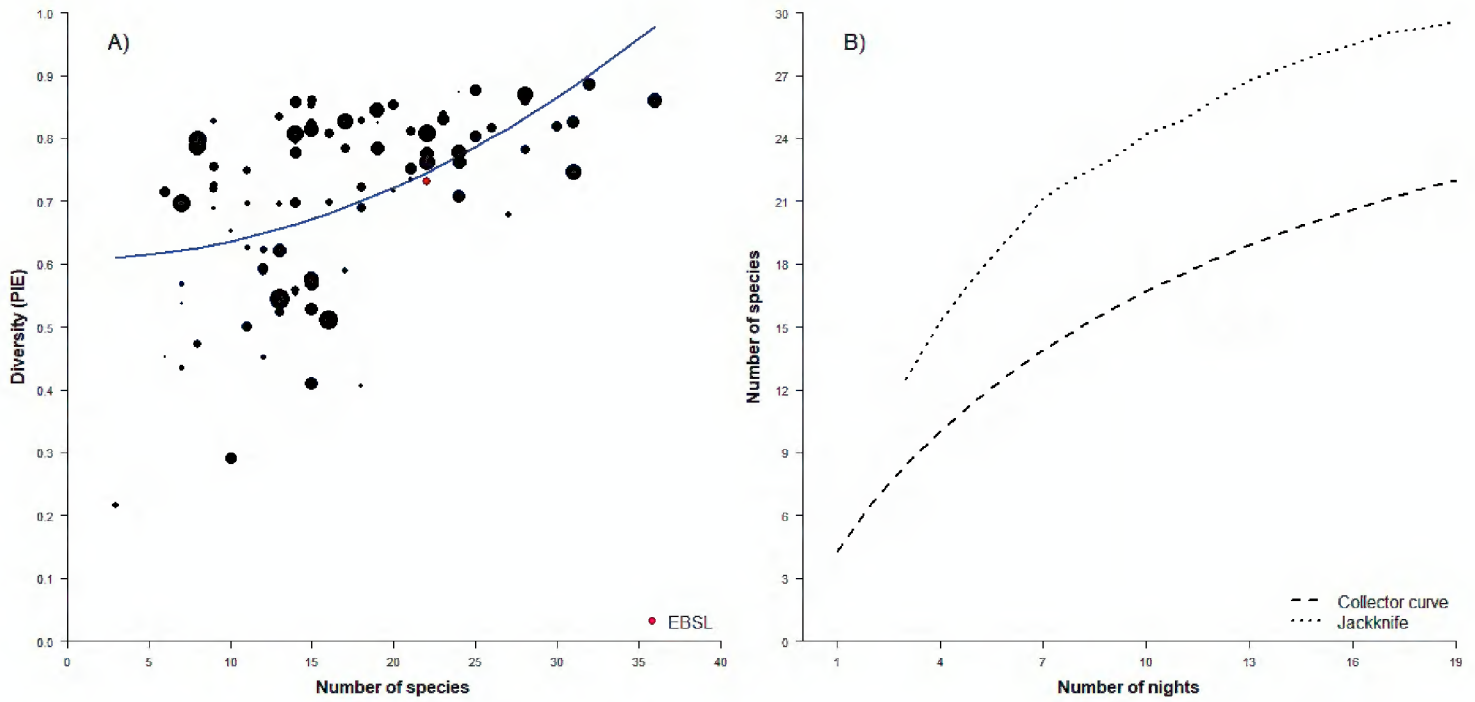

Figure 2. Bat assemblages in an area of Atlantic Forest, southeastern Brazil. **A.** Species richness and PIE values from 90 inventories of bats. EBSL is highlighted by the red dot. Area of the locality is represented by the dot size. Blue line depicts a fitted model of expected PIE values due to species richness. See Suppl. material 1: Table S1 for details. **B.** Species accumulation curves based on first-order Jackknife richness estimator. Dashed line are the accumulated average number of species, after randomizations, and dotted line depicts the number of species estimated by Jackknife.

fauna at EBSL remains partially undiscovered. It was demonstrated that species richness tends to stabilize, for the Phyllostomidae at the Atlantic Forest, around 1 000 captures (Bergallo et al. 2003). We had 194 phyllostomid captures in our sampling, so these results are expected.

We recorded a few noteworthy species in this study. Gleaning animalivores feed mainly on animals, though a few species ingest more fruit and pollen than animals, such as *Phyllostomus hastatus* and *Phyllostomus discolor* (Giannini and Kalko 2004). Members of this guild are found more often in forested rather than in deforested areas, and are considered suitable indicators of habitat disruption caused by deforestation (Fenton et al. 1992). We captured four species of gleaning animalivores – *Micronycteris microtis*, *Mimon bennettii*, *Phyllostomus discolor*, and *Trachops cirrhosus* – at EBSL, each represented by a single individual, with a fifth (*Micronycteris minuta*) known to occur by voucher specimens. This can be explained by the relatively low sampling size of our collection, but it may also reflect an impact at EBSL. In fact, it has vegetation of late stages of ecological succession, with signs of anthropic disturbance in areas of old agriculture activity (Mendes and Padovan 2000).

We captured a single *Lonchophylla peracchii* specimen, already known to occur in the Espírito Santo state (Pimenta et al. 2010). Until recently, this species was lumped with *Lonchophylla bokermanni*, but a morphological analysis revealed the existence of two species, with Atlantic Forest populations reassigned to *Lonchophylla peracchii* (Dias et al. 2013, Cláudio et al. 2018). This is the one of the Atlantic Forest endemic bat species, among *Eptesicus taddeii*, *Histiotus alienus*, *Lasiurus ebenus* and *Myotis levis*

(Delgado-Jaramillo et al. 2020). While *Lonchophylla peracchii* is classified as Least Concern by the IUCN (Solari 2017), it is considered as Data Deficient in the last assessment of the state of Espírito Santo fauna (Costa et al. 2019). Another species with that classification registered in the present study is *Micronycteris microtis*. Furthermore, *Myotis ruber* is listed as a Near Threatened by the IUCN (Solari 2019).

Conclusion

Our research describes an assemblage of bats within the Atlantic Forest of Espírito Santo, southeast Brazil, in one of the oldest Brazilian conservation areas. Despite the dominance by common frugivorous species, the EBSL has a medium to high diversity, and we expect that further inventories will find more species.

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Appendix 1

Vouchers deposited at the Mammalian Collection of Universidade Federal do Espírito Santo (UFES-MAM), Brazil.

Anoura caudifer: 3964, 3965, 3966. *Anoura geoffroyi*: 3260, 3261, 3262, 3263, 3264, 3265. *Artibeus fimbriatus*: 3827. *Artibeus lituratus*: 4376, 4379, 4532. *Artibeus obscurus*: 4377, 4378, 4380. *Carollia perspicillata*: 3716, 3717, 3718, 3719, 3720, 3721, 3722, 3723, 3724, 3725, 3726, 3727, 3730. *Dermanura cinerea*: 4150, 4248, 4381. *Desmodus rotundus*: 4287. *Lonchophylla peracchii*: 3225. *Micronycteris microtis*: 3943. *Mimon bennettii*: 3178. *Phyllostomus hastatus*: 3525. *Platyrrhinus recifinus*: 4311. *Rhinophylla pumilio*: 4087, 4088, 4089, 4090, 4091, 4092, 4093. *Sturnira lilium*: 3547. *Sturnira tildae*: 4206, 4207. *Trachops cirrhosus*: 3776. *Vampyressa pusilla*: 4210, 4211. *Eptesicus brasiliensis*: 3269. *Myotis riparius*: 4239, 4240, 4241, 4242, 4243. *Myotis ruber*: 4041.

Supplementary material 1

Table S1. Locality, species richness, and calculated Shannon, Pielou and PIE diversity indexes from 119 bat species inventories at the Atlantic Forest, southeastern Brazil

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Data type: species data

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